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Histamine *N*-methyl transferase: inhibition by drugs

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1 Histamine *N*-methyl transferase activity was measured in samples of human liver, brain, kidney, lung and intestinal mucosa. The mean (± s.d.) rate (nmol min^{-1} mg^{-1} protein) of histamine *N*-methylation was 1.78 ± 0.59 (liver, $n = 60$), 1.15 ± 0.38 (renal cortex, $n = 8$), 0.79 ± 0.14 (renal medulla, $n = 8$), 0.35 ± 0.08 (lung, $n = 20$), 0.47 ± 0.18 (human intestine, $n = 30$) and 0.29 ± 0.14 (brain, $n = 13$).

2 Inhibition of histamine *N*-methyl transferase by 15 drugs was investigated in human liver. The IC_{50} for the various drugs ranged over three orders of magnitude; chloroquine was the most potent inhibitor.

3 The average IC_{50} values for chloroquine were 12.6, 22.0, 19.0, 21.6 μM in liver, renal cortex, brain and colon, respectively. These values are lower than the Michaelis-Menten constant for histamine *N*-methyltransferase in liver (43.8 μM) and kidney (45.5 μM). Chloroquine carried a mixed non-competitive inhibition of hepatic histamine *N*-methyl transferase. Some side-effects of choroquine may be explained by inhibition of histamine *N*-methyl transferase.

Keywords histamine *N*-methyl transferase man tissue distribution enzyme inhibition antimalarials

Introduction

Treatment with chloroquine, a potent inhibitor of histamine *N*-methyl transferase, is often associated with gastrointestinal, neurological and dermatological side-effects (Martindale, 1989). Beaven & Shaff (1979), Mehler *et al.* (1952), Schayer (1952) and Tachibana *et al.* (1986) have observed that histamine *N*-methyl transferase is quantitatively more important than diamino oxidase in the metabolism of histamine in different animal tissues. Drugs inhibit to different extents the activity of histamine *N*-methyl transferase in animal tissues (Barth & Lorenz, 1978; Barth *et al.*, 1975; Beaven, 1982; Beaven & Shaff, 1979; Cumming *et al.*, 1990; Duch *et al.*, 1978, 1980, 1984; Futo *et al.*, 1990; Harle & Baldo, 1988; Tachibana *et al.*, 1988; Thithapandha & Cohn, 1978) and chloroquine is among the most potent inhibitors (Tachibana *et al.*, 1988). Currently little is known about the inhibition of human histamine *N*-methyl transferase by drugs. Van Loon *et al.* (1985) observed that amodiaquine is a non-competitive inhibitor of histamine methylation in human erythrocytes.

Three methyl transferases have been described and they are defined with respect to the functional moiety of the substrates that are methylated (*O*-methyl transferase, *N*-methyl transferase and S-methyl transferase). These enzymes share the co-substrate S-adenosyl-L-methionine (SAM) which is the methyl donor (Asano *et al.*, 1984; Zappia *et al.*, 1969).

The tissue distribution of S-methyl transferases has been described in man (Pacifici *et al.*, 1991a,b,c), whereas little is known of the distribution of *N*-methyl transferase (Hesterberg *et al.*, 1984). Two isoenzymes of *N*-methyl transferase, referred to as *N*-methyl transferase A and B, have been isolated from rabbit liver (Ansher & Jakoby, 1990). Histamine *N*-methyl transferase seems to differ from transferases A and B in its specificity (Brown *et al.*, 1959; Thithapandha & Cohn, 1978). We describe the distribution of histamine *N*-methyl transferase in human tissues and its inhibition by drugs.

Methods

Chemicals

Labelled adenosyl-L-methionine-S-[methyl (^{14}C)] (specific activity 58 mCi $mmol^{-1}$, radiochemical purity = 98.6, SAM) was obtained from Du Pont NEN Research Products (Florence, Italy). Dithiothreitol (DTT), histamine, pargyline, Tris (Tris-hydroxymethyl aminomethane), glycine and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Potassium phosphate monobasic, potassium phosphate dibasic, sodium hydroxide, toluene, isoamyl

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alcohol, perchloric acid and dimethylsulphoxide (DMSO) were purchased from Carlo Erba (Milan, Italy). The following drugs were gifts: chloroquine HCl and hydroxychloroquine HCl (Bayer, Milan, Italy), chloroguanil-HCl, primaquine phosphate, proguanil HCl and pyrimethamine, (ICI, Macclesfield, UK), promethazine, chlorpromazine and trimethoprim (Farmitalia Carlo-Erba, Milan, Italy), tripelennamine HCl (Ciba-Geigy, Saronno, Italy), metoclopramide HCl (Sharper, Milan, Italy), ranitidine HCl (Guidotti, Pisa, Italy), cimetidine (Smith Kline & French, Milan, Italy) and clomiphenecitrate (Lepetit, Milan, Italy).

Biological material

Wedge liver specimens were obtained from 23 men and 37 women (aged between 22 and 80 years) undergoing cholecystectomy. Material surplus to that required for histological analysis was made available for our study. The liver specimens included in the study had normal cell architecture. Specimens of human lung were obtained from 2 women and 18 men (aged between 27 and 74 years) undergoing lobectomy for removal of a tumour. A specimen of normal tissue adjacent to the tumour was made available for our study. Of the 20 lung tissue donors, 7 were smokers and 13 were ex-smokers. An ex-smoker was defined as an individual who ceased smoking at least 6 months before surgery. Specimens of human kidney were obtained from three women and five men (aged between 32 and 75 years) undergoing renectomy for removal of a tumour, the volume of which was limited and left intact at least 2/3 of the architecture of the kidney. The renal cortex was isolated from the medulla immediately after resection of the tissue. Specimens of normal kidney adjacent to the tumour were made available for our study. Samples of intestinal mucosa were obtained from 14 men and 16 women aged between 22 and 74 years. The intestine was surgically removed because of a tumour. The mucosa was isolated from a normal part of the intestine adjacent to the tumour within 10 min after resection of the tissue. Ten of the gut donors underwent right hemicholectomy and the mucosa was isolated from the last portion of the ileum and the first part of the ascending colon. Specimens of human brain were obtained at surgery for removal of a tumour. Part of the normal cerebral tissue adjacent to the tumour was made available for our study. Brain donors were three women and nine men aged between 52 and 74 years. A woman 52 years old (frontal cortex donor) received valproic acid (1 g day^{-1}) for the 2 weeks preceding surgery. A man 66 years old (occipital cortex donor) was treated with phenobarbitone (100 mg day^{-1}) over several years, whereas a woman 63 years old (parietal cortex donor) was under chronic treatment with anti-depressants. A specimen of frontal cortex and subcortex was obtained from a 60 year old woman. Tissue specimens were frozen immediately and stored at −80° C.

Subcellular isolation

Specimens of liver and brain (0.2–0.5 g) and of kidney, lung and intestinal mucosa (2 g) were homogenized in 5 volumes 0.25 M sucrose. Liver and brain tissue was dispersed using a glass-Teflon homogenizer. Samples of kidney, lung and intestinal mucosa were homogenized using a Polytron S-10 (Kinematica, Luzern, Switzerland) at power setting 4 (3 × 5 s). The homogenates were re-homogenized using a glass-Teflon homogenizer and then filtered through gauze. The homogenates were then centrifuged at 12,000 *g* for 15 min and the resultant supernatants were centrifuged again at 105,000 *g* for 1 h. The final supernatants were divided into aliquots, stored at −80° C, and investigated as the cytosolic fraction.

Histamine N-*methyl transferase assay*

The activity of histamine *N*-methyl transferase was measured by a radiometric assay based on the use of [^{14}C]-SAM. The method was a combination of those described by Van Loon *et al.* (1985) and Harvima *et al.* (1988). The final incubation volume was 100 μl and the assay was performed in Eppendorf tubes. The incubation mixture consisted of 200 μM potassium orthophosphate buffer (pH 8.5), 300 μM histamine, 12 μM SAM (150,000 counts min^{-1}), 1 mM pargyline and an aliquot of the cytosolic fraction diluted 100-fold in a solution containing 0.66 mg BSA and 1.56 mg DTT ml^{-1} of 5 mM potassium orthophosphate buffer (pH 7.4). The final concentration of the cytosolic protein in the incubation mixture ranged from 10–25 μg ml^{-1} incubate. The reaction was started by the addition of histamine, carried out at 37° C for 30 min and then stopped by the addition of 20 μl of 2.5 M perchloric acid. Aliquots (20 μl) of 10 M sodium hydroxide and 1 ml toluene/isoamyl alcohol (4:1 v/v) were added and the tubes were rotated at 40 rev min^{-1} for 20 min and then centrifuged at 800 *g* for 5 min. An aliquot (0.5 ml) of the organic phase was transferred to scintillation vials containing 3 ml scintillator (Emulsifier Scintillator 299, Packard, Milan, Italy). The radioactivity was measured using a Beckman model LS 1701 scintillation counter. Each sample was assayed in duplicate accompanied by two controls without histamine. The activity of histamine *N*-methyl transferase was measured after correction for control values on the basis of the specific activity of [^{14}C]-SAM. For the kinetic study, the concentration of histamine ranged between 18.75 and 300 μM and the concentration of SAM was kept constant at 12 μM. Alternatively, the concentration of SAM ranged between 0.75 and 12 μM and the concentration of histamine was kept constant at 300 μM.

The inhibition by drugs of histamine *N*-methylation was investigated in a pilot study using three concentrations of drug covering a 100-fold range. After identification of the appropriate range of inhibitor concentrations, a final study was performed with 5 to 7 concentrations of the inhibitor, each being one half of the next one. All drugs were tested with the same liver samples whose donors were one woman 42 years old and two men 39 and 69 years old. The inhibitors were dissolved in water with the exception of promethazine, chlorpromazine, cimetidine, trimethoprim and pyrimethamine, which were dissolved in DMSO and added in a 5 μl volume to the incubation mixture. Controls contained DMSO only, which had no effect on the enzyme activity. The type of inhibition caused by chloroquine was determined by studying varying concentrations of histamine in the presence or absence of chloroquine.

Protein concentration was measured as described by Lowry *et al.* (1951).

Calculations

Values of K_m and V_{max} were estimated from Eadie-Hoffstee plots and calculated using the ENZPACK program (Elsevier Biosoft). IC_{50} values were computed using the program 'Dose, effect, binding and kinetics' (Elsevier Biosoft). Differences in the activity of histamine *N*-methyl transferase between renal cortex and medulla and between ileum and colon were assessed by Student's *t*-test for paired data.

Results

The otpimal assay conditions for the methylation of histamine were determined initially. The reaction was linear over 40 min of incubation and, at least, up to a cytosolic protein concentration of 50 μg ml^{-1} incubation. The optimum pH was 8.5. The activity of histamine methylation was measured in 60 specimens of human liver, in eight specimens of human renal cortex and medulla, in 20 specimens of human lung and in 30 specimens of intestinal mucosa (Figure 1). Histamine *N*-methyl transferase activity was not related to the age or sex of the tissue donor. Hepatic histamine *N*-methyl transferase activity showed a 4-fold range with a coefficient of variation of 33%. Smoking did not affect the activity of histamine *N*-methyl transferase in human lung. Histamine *N*-methyl transferase activity was significantly ($P < 0.01$) higher in renal cortex than medulla, whereas it was not different in ileum and colon. Table 1 summarizes the activity of histamine *N*-methyl transferase in different areas of the human brain. The rate of histamine *N*-methylation was similar in the various brain specimens investigated including the specimens of frontal cortex (F 52) and occipital cortex (M 66) whose donors were treated with valproic acid and phenobarbitone. Histamine *N*-methyl transferase activity in cortex was 50% greater than in medulla.

Enzyme kinetics were studied in three specimens of liver and kidney and Table 2 summarizes the kinetic parameters. The Michaelis-Menten constant measured at varying concentrations of histamine or SAM was similar in liver and kidney, whereas the maximum rate of reaction was about 50% higher in liver than kidney.

Figure 2 shows the effects of 15 drugs on the methylation rate of histamine. Table 3 summarizes the IC_{50} values. The most potent inhibitor was chloroquine, the IC_{50} of which was 2 to 3 times lower than the K_m for histamine *N*-methyl transferase. The inhibitory effect of chloroquine observed in liver was similar to that in renal cortex, brain and intestinal mucosa (Table 4). The mechanism of inhibition by chloroquine was studied in three liver samples. The results of a representative experiment are shown in Figure 3 and the kinetic parameters for all livers investigated are summarized in Table 5. Chloroquine increased the Michaelis-Menten constant and decreased the maximum velocity of the reaction, indicating a non-competitive mixed inhibition.

Discussion

Histamine *N*-methyl transferase was measurable in all tissue specimens assayed. Liver samples catalysed the methylation of histamine at a rate 50% higher than those from the kidney, whereas the average activity in intestinal mucosa, lung and brain was 3 to 5 times lower than the hepatic activity. Thus, histamine *N*-methyl

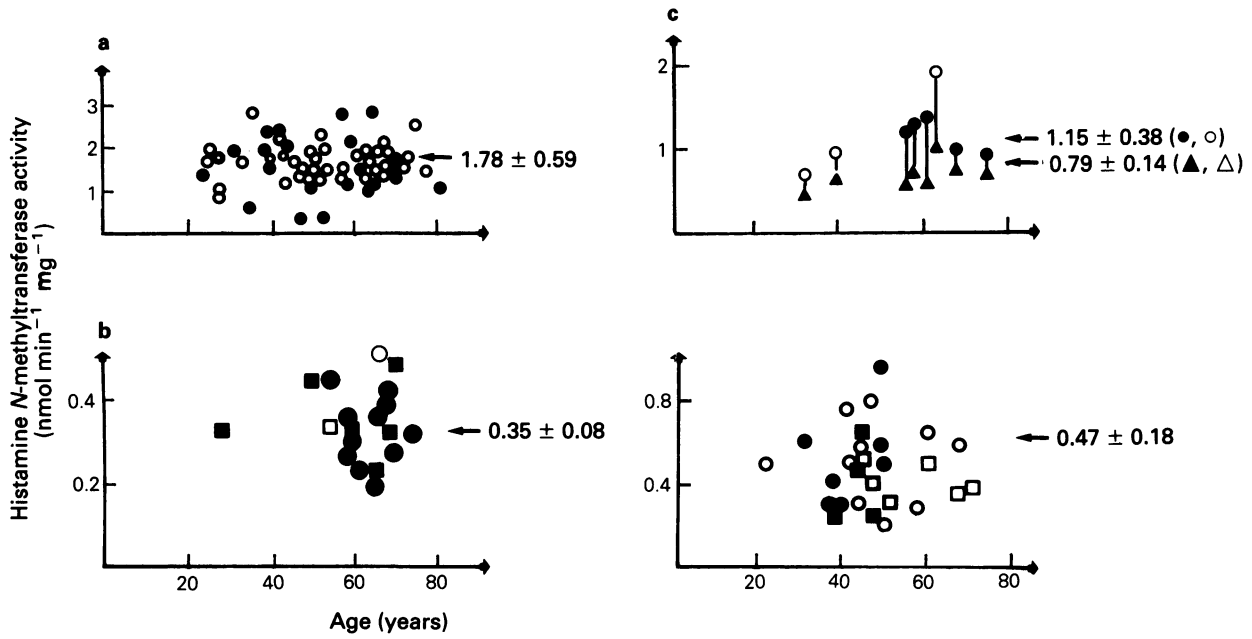

Figure 1 Histamine *N*-methyl transferase activity in a) human liver ($n = 60$), b) lung ($n = 20$), c) kidney ($n = 8$) and d) intestinal mucosa ($n = 30$). b) Squares and circles refer to smokers and ex-smokers, respectively. c) Circles and triangles refer to renal cortex and medulla, respectively. d) Circles and squares refer to ileum and colon, respectively. a,b,c,d) Filled and unfilled symbols refer to men and women, respectively. Numbers refer to means ± s.d. of enzyme activity.

Table 1 Activity of histamine *N*-methyl transferase in different areas of the human brain

Sex	*Age (years)*	*Brain specimen*	*Histamine* N-*methyl transferase activity (nmol* min^{-1} mg^{-1}*)*
M	55	Frontal cortex	0.14
F	52	Frontal cortex	0.29
M	74	Frontal cortex	0.25
F	60	Temporal cortex[a]	0.32
M	47	Temporal cortex	0.73
M	54	Temporal cortex	0.24
M	58	Temporal cortex	0.21
F	61	Temporal cortex	0.34
M	63	Temporal cortex	0.25
F	60	Temporal subcortex[a]	0.23
M	63	Parietal cortex	0.19
M	66	Occipital cortex	0.21
M	56	Cerebellum cortex	0.36
Mean ± s.d.			0.29 ± 0.14

[a]Cortex and subcortex were from the same donor.

Table 2 Kinetic parameters for histamine *N*-methyl transferase in human liver and kidney. Variable histamine: histamine concentration 18.75–300 μM; constant SAM 12 μM. Variable SAM: SAM concentration 0.75–12 μM; constant histamine 300 μM

		Liver			
		Variable histamine		*Variable SAM*	
Sex	*Age (years)*	K_m[a]	V_{max}[b]	K_m[a]	V_{max}[b]
M	39	37.8	3.28	4.95	2.95
F	42	41.8	3.58	4.57	3.08
M	69	52.0	4.11	7.05	3.08
Mean ± s.d.		43.9 ± 6.0	3.66 ± 0.34	5.52 ± 1.09	3.04 ± 0.06
		Kidney			
		Variable histamine		*Variable SAM*	
Sex	*Age (years)*	K_m[a]	V_{max}[b]	K_m[a]	V_{max}[b]
F	63	43.3	2.62	3.28	2.52
M	61	41.4	1.65	3.02	1.63
M	57	51.9	1.53	4.23	1.52
Mean ± s.d.		45.5 ± 4.6	1.93 ± 0.49	3.51 ± 0.52	1.89 ± 0.45

[a]μM; [b]nmol min^{-1} mg^{-1}.

Table 3 IC_{50} values for the inhibition of histamine *N*-methyl transferase by different drugs (mean ± s.d. values for three livers)

Drug	IC_{50} *(*10^{-6} *M)*
Chloroquine	13.5 ± 4.1
Hydroxychloroquine	22.6 ± 7.8
Pyrimethamine	25.5 ± 7.1
Promethazine	224 ± 24
Chloroguanil	373 ± 107
Proguanil	490 ± 82
Tripelennamine	515 ± 59
Metoclopramide	657 ± 28
Ranitidine	1350 ± 96
Primaquine phosphate	1470 ± 17
Ranitidine	1350 ± 80
Cimetidine	3086 ± 448
Trimethoprim	8570 ± 700
Chlorpromazine	Not measurable
Clomiphene	Not measurable

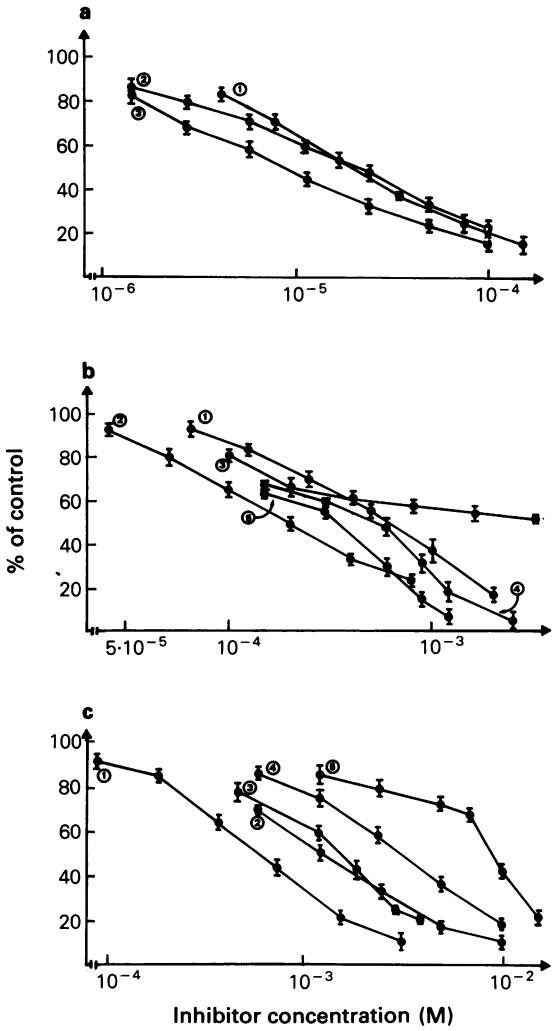

Figure 2 The effects of various drugs on the activity of hepatic histamine *N*-methyl transferase. a) The circled numbers 1, 2 and 3 refer to pyrimethamine, hydroxychloroquine and chloroquine, respectively. b) The circled numbers 1, 2, 3, 4 and 5 refer to tripelennamine, promethazine, chlorpromazine, proguanil and chlorproguanil, respectively. c) The circled numbers 1, 2, 3, 4 and 5 refer to metoclopramide, ranitidine, primaquine, cimetidine and trimethoprim, respectively. Points represent average data for three livers; vertical bars indicate 2 × s.d. of the mean.

Table 4 Inhibition of histamine *N*-methyl transferase in different human tissues by chloroquine

					IC_{50} values (μM)						
Sex	*Age (years)*	*Liver*	*Sex*	*Age (years)*	*Renal cortex*	*Sex*	*Age (years)*	*Brain*	*Sex*	*Age (years)*	*Colon*
M	39	19.5	F	63	26.9	M	47	18.2	M	69	16.2
F	64	10.8	M	61	23.6	M	56	25.4	F	67	22.1
F	43	9.7	M	57	21.8	F	61	17.5	F	62	16.2
F	42	10.3	M	55	15.7	F	62	14.9	M	51	32.1
Mean		12.6			22.0			19.0			21.6
± s.d.		4.6			4.6			4.5			7.5

Brain: the cases M 47 and M 61 refer to temporal cortex. The cases M 56 and F 62 refers to cerebellum cortex and frontal subcortex, respectively.

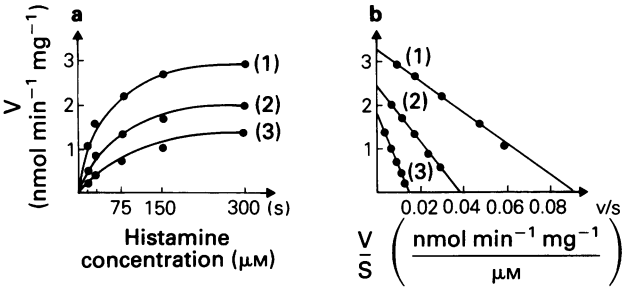

Figure 3 Effect of different concentrations of chloroquine on the histamine *N*-methyl transferase activity in a liver sample from a single donor (male, 39 years old). The numbers 1, 2 and 3 refer to the concentrations of chloroquine in the incubation mixture (0, 6.25 and 25 μM, respectively). a) Rate *vs* substrate concentration plot and b) Eadie-Hoffstee plots.

transferase has in keeping with previous data from animals (Beaven, 1982) and man (Hesterberg *et al.*, 1984), ubiquitous distribution in the body.

Since histamine is a neurotransmitter it was of interest that brain had the lowest activity of histamine *N*-methyl transferase. The highest concentrations of histamine have been found in the hypothalamus and area postrema (Bloom, 1990), which are not surgically removable. Thus, we do not know whether the activity of histamine *N*-methyl transferase in these areas is different from that in the areas that were assayed. Treatment with valproic acid and phenobarbitone appeared to have no effect on human brain histamine *N*-methyl transferase, whereas, in the same specimens, these drugs greatly induced the rate of 1-naphthol glucuronidation (Viani *et al.*, 1990).

The Michaelis-Menten constant for histamine was similar in human liver and kidney and it was also similar to that for guinea pig brain (Thithapandha & Cohn, 1978). Francis *et al.* (1980) reported a K_m value for human skin one tenth of that shown for liver and kidney in the present study. These different K_m values might reflect the presence of endogenous inhibitors or a tissue-dependent affinity for the physiological substrate of histamine *N*-methyl transferase.

The interaction of drugs with histamine *N*-methyl transferase has been described in rodents (Barth *et al.*, 1975; Barth & Lorenz, 1978; Beaven & Shaff, 1979; Duch *et al.*, 1978, 1980, 1984; Harle & Baldo, 1988; Tachibana *et al.*, 1988; Thithapandha & Cohn, 1978). Van Loon *et al.* (1985) observed that amodiaquine inhibited human erythrocyte histamine *N*-methyl transferase, the only investigation with human material. For some of the drugs studied, such as the antimalarials chloroquine, hydroxychloroquine and pyrimethamine, the IC_{50} values were lower than the plasma drug concentrations associated with therapeutic effect. The IC_{50}s for trimethoprim, cimetidine, primaquine phosphate, ranitidine, metoclopramide and tripelennamine were higher than the expected therapeutic concentrations and it is unlikely that these drugs inhibit the *N*-methylation of histamine *in vivo*. Chloroquine and pyrimethamine were potent inhibitors of histamine *N*-methyl transferase despite their different molecular structures. Trimethoprim was not an inhibitor although its chemical structure resembles that of pyrimethamine.

The inhibitory effect of chloroquine was similar in liver, kidney, brain and intestinal mucosa. It is possible that some of the many side-effects of chloroquine are related to increase in tissue histamine consequent to inhibition of histamine *N*-methyl transferase. Thus, chloroquine and amodiaquine, potent inhibitors of histamine *N*-methyl transferase, cause side-effects at therapeutic dosages, whereas proguanil, chloroguanil, primaquine and trimethoprim are well tolerated at normal therapeutic doses (Martindale, 1989).

Table 5 Kinetic parameters for histamine *N*-methyl transferase in the presence and absence of chloroquine. The concentration of histamine ranged between 18.7 μM and 300 μM, whereas the concentration of SAM was constant at 12 μM

		Control		*Chloroquine 6.25 μM*		*Chloroquine 25 μM*	
Sex	*Age (years)*	K_m[a]	V_{max}[b]	K_m[a]	V_{max}[b]	K_m[a]	V_{max}[b]
F	35	26.0	4.77	78.0	4.00	107	2.47
M	39	37.8	3.28	64.9	2.44	138	1.96
M	64	52.0	4.11	86.3	2.67	301	2.45
Mean		38.6	4.05	76.4	3.04	182	2.29
± s.d.		10.6	0.61	8.81	0.69	85.1	0.23

[a]μM; [b]nmol min^{-1} mg^{-1}.

The non-competitive mixed inhibition of histamine *N*-methyl transferase by chloroquine in human liver is in accord with the effect of amodiaquine in human erythrocytes (Van Loon *et al.*, 1985). In contrast, all of the inhibitors tested including chloroquine competitively inhibit histamine *N*-methyl transferase in guinea pig skin (Tachibana *et al.*, 1988). Chloroquine as well as the other inhibitors studied do not undergo methylation. Therefore, the inhibition cannot be explained by competition for substrates. It remains to be seen whether chloroquine inhibits histamine *N*-methyl transferase only or if such an inhibition also occurs with the other isoenzymes catalysing the methylation of amines.

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